The association between liver fat content and plasma metabolite profiles in fasting and postprandial states: an integration of a cohort study and a randomized controlled trial

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The association between liver fat content and plasma metabolite profiles in fasting and postprandial states: an integration of a cohort study and a randomized controlled trial

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Abstract

Background Postprandial metabolic impairments play a key role in the pathophysiology of cardiometabolic diseases. While liver fat content has been linked to distinct fasting metabolite profiles, its relationship with postprandial metabolite profiles remains unexplored. In this study, we aimed to (1) examine to what extent liver fat content is associated with the postprandial metabolomic profile beyond fasting metabolites; and (2) investigate whether diet-induced changes in liver fat content are associated with changes in plasma metabolites identified in objective 1.

Methods In a subpopulation ($n = 1986$) of an existing cohort study and a 12-week dietary intervention study ($n = 80$), liver fat content was measured by proton magnetic resonance spectroscopy and categorized as low (< 2.5%), middle (2.5–5.5%), or high (> 5.5%). In the cohort study, plasma metabolomic profiles were quantified by NMR spectroscopy at fasting (T_0) and 150 min after a mixed meal (T_{150}). We examined associations between liver fat content and plasma metabolites at T_0, T_{150} and postprandial response (ΔT_{150}-T_0) using multivariate linear regression. In the intervention study, plasma metabolomic profiles were quantified at fasting (T_0) and at multiple postprandial time points (120, 240, and 360 min) following a mixed meal, both before and after the intervention. We further examined associations between liver fat content and plasma metabolites at T_0, and postprandial response (incremental area under the curves [iAUCs]) and explored associations between diet-induced changes in liver fat content and changes in identified metabolites at fasting and postprandial responses (iAUCs).

Results High liver fat group was characterized by higher fasting and postprandial levels of triglycerides, all VLDL and the small LDL/HDL subclasses, ApoB, fatty acids, glycoprotein acetyls, and BCAAs, and lower medium/larger HDL subclasses, and acetate compared to the low liver fat group. In the high vs. low liver fat group, postprandial responses

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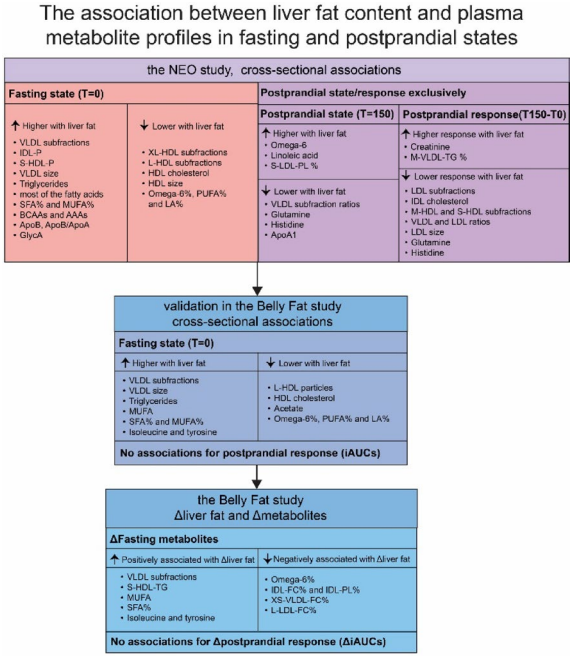
of cholesterol content of S-LDL, IDL, and S-HDL, glutamine and histidine, omega-3% and DHA % were lower. Diet-induced reductions in liver fat were associated with reductions in 40 fasting plasma metabolites, including VLDL-TG, tyrosine, isoleucine, fatty acid ratios, and most of the VLDL subclasses.

Conclusions Postprandial metabolomic profiling revealed additional associations between liver fat content and plasma metabolites beyond fasting measures, particularly in lipoprotein cholesterol and fatty acid composition. Diet-induced reductions in liver fat were associated with favorable changes in fasting metabolites, but not postprandial metabolite responses. Future studies with harmonized postprandial assessment are needed to further elucidate the postprandial observations and the underlying mechanisms.

Trial registration The trials in this study were registered at clinicaltrials.gov as NL21981.058.08/P08.109 and NCT02194504.

Keywords Liver fat, Metabolomics, Postprandial metabolism, Lipid metabolism, Lipoproteins

Graphical abstract



Background

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a condition characterized by excess fat accumulation in the liver. MASLD results from an imbalance in lipid metabolism, enhanced hepatic *de novo* lipogenesis, impaired export of hepatic triglycerides, and relative defects in mitochondrial beta-oxidation [1–3]. Over the past decades, the prevalence of MASLD has notably increased worldwide, with MASLD currently affecting about 30% of the world's adult population [4]. MASLD is strongly associated with obesity, insulin resistance, dyslipidaemia, cardiovascular disease (CVD) and type 2 diabetes (T2D) [5, 6].

Liver fat content has been reported to be associated with numerous fasting metabolites and biochemical pathways [1, 7, 8]. Previous studies have shown that MASLD present different lipid and lipoprotein profiles, including higher plasma saturated (SFAs) and monounsaturated FAs (MUFAs), as well as higher very low-density lipoprotein triglycerides (VLDL-TG), small high-density lipoprotein particles (S-HDL-P), and glycoprotein acetyls (GlycA), whereas larger HDL particles were inversely associated with liver fat [9–12]. In addition, plasma amino acids, especially branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs), were consistently found to be elevated in MASLD [13, 14]. However, most studies have focused on metabolite profiles in the fasting state. As the liver plays a crucial role in postprandial metabolism, investigating postprandial responses to metabolic challenges may provide new insights into metabolic alterations associated with liver fat accumulation. Some studies demonstrated associations between MASLD and several postprandial markers, including lower postprandial lysosomal acid lipase and

higher diacylglycerol in MASLD compared to healthy controls [15, 16]. To our knowledge, other postprandial metabolites have not been investigated in relation to liver fat or MASLD.

Furthermore, sex differences have been reported in MASLD. Men are more likely to develop steatosis at an earlier stage, whereas women, particularly after menopause, show a higher susceptibility to fibrosis progression [17, 18]. Despite established sex differences reported in lipid metabolism and liver disease progression [19–21], sex differences in associations between liver fat content and metabolic profiles, especially postprandial metabolic profiles, remain unexplored.

To explore liver fat content-associated metabolic profiles, we aimed to investigate associations between liver fat content and fasting as well as postprandial plasma metabolite profiles in an observational cohort study of middle-aged men and women ($n = 1986$), and validate these in a 12-week dietary intervention study ($n = 80$) with 25% energy restriction (ER) in which we previously showed a reduction in body weight up to 8.4 kg and a concurrent reduction in liver fat content up to 3.9% [22]. For the present study, we aimed to (1) explore whether postprandial plasma metabolomic profiles reveal additional associations with liver fat content beyond fasting status; (2) assess whether diet-induced changes in liver fat are associated with changes in fasting and postprandial plasma metabolite levels, leveraging the intervention study; and (3) investigate potential effect modification of liver fat-metabolite associations by sex.

Materials and methods

Study design and populations

The Netherlands epidemiology of obesity (NEO) study

The NEO study is a prospective cohort study designed to investigate pathways that lead to obesity-related diseases. The present analysis is cross-sectional and based on baseline measurements. The study was approved by the medical ethical committee of the Leiden University Medical Center (LUMC) and registered at clinicaltrials.gov under number NL21981.058.08/P08.109. All participants have given written informed consent. Details of the study design can be found elsewhere [23] and in the supplemental materials. Briefly, participants aged between 45 and 65 years with a self-reported body mass index (BMI) of 27 kg/m^2 or higher living in the greater area of Leiden were invited for a baseline visit between September 2008 and September 2012. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited, irrespective of their BMI. Demographic, lifestyle and clinical information were collected via a general questionnaire. At baseline, after an overnight fast, anthropometric measures were taken and a mixed-meal test was performed. Blood samples were drawn at fasting and 150 min after the consumption of a liquid mixed meal (400mL, 600 kcal, with 16% of energy [En%] derived from protein, 50 En% carbohydrates, and 34 En% fat). Plasma samples were then separated, aliquoted and stored at − 80 °C for future analyses.

Of the 6671 participants, liver fat content was measured in 2083 individuals, who were included in the analysis. An additional 205 participants were excluded for the following reasons: (1) 95 participants had more than 40% missingness for metabolomics measurements; (2) 2 participants were not overnight fasted; (3) 108 participants violated the meal challenge protocol. This resulted in 1986 participants included in analyses with fasting metabolites, and 1878 participants included in the analyses with postprandial metabolites (Fig. 1).

The belly fat study

To validate the associations in an independent population and examine the associations between diet-induced changes in (Δ) liver fat content and changes in (Δ) fasting and postprandial plasma metabolite levels, data from a parallel-designed, 12-week 25% energy-restricted (ER) dietary intervention study (the Belly Fat Study [22]) were used. This study was approved by the Medical Ethics Committee of Wageningen University and registered at www.clinicaltrials.gov under NCT02194504. A subset of participants ($n = 80$ of 110) had both plasma metabolomics and liver fat content data available. Methodological details can be found elsewhere [22] and in the supplemental materials. Briefly, men and women aged 40–70 years with abdominal obesity (BMI > 27 kg/m^2 or a waist circumference > 88 cm [women] or > 102 cm [men]) were randomized to one of three 12-week diets: 25% ER high-nutrient-quality diet ($n = 33$); 25% ER low-nutrient-quality diet ($n = 28$); or control group, where participants maintained their habitual diet ($n = 19$). At baseline and after 12 weeks, blood samples were collected after overnight fast and 120, 240, and 360 min after consumption of a high-fat, high-glucose liquid mixed meal (400 ml, 916 kcal, with 8% of En% derived from protein, 33 En% carbohydrates, and 59 En% fat) [24]. Plasma samples were then separated, aliquoted and stored at − 80 °C for future analyses.

Data collection

Assessment of liver fat content

Liver fat content was assessed by proton magnetic resonance spectroscopy (^{1}H-MRS) in the NEO study. Details have been reported previously [25]. Participants were categorized into three groups based on liver fat content: low liver fat group ($n = 568$) (liver fat content < 2.5%), middle liver fat group ($n = 490$) (liver fat content 2.5–5.5%) and high liver fat group ($n = 928$) (liver fat content > 5.5%). The cutoff of 5.5% was based on previous studies in adults

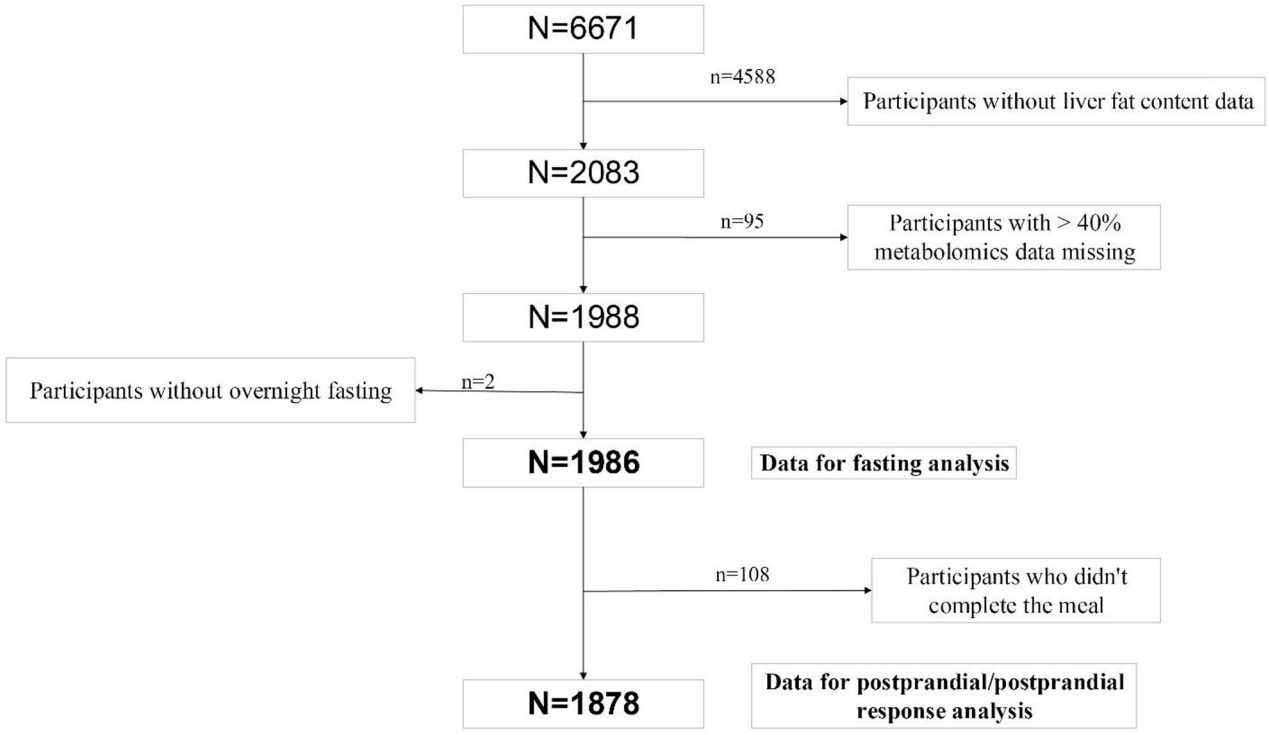


Fig. 1 The flowchart of data used from the NEO study. Among the 6671 participants in the NEO study, 2083 of them had liver fat content measured. Among those participants, 95 participants had a greater than 40% missingness for metabolomics measurements, and 2 participants were not overnight fasted. These data were removed for fasting analysis. Furthermore, 108 participants of the remaining were removed for postprandial/postprandial response analysis due to incomplete meals

using ^{1}H-MRS [26], and to avoid potential biases introduced by single cutoff selection and enable comparison across gradients of liver fat content, we further categorized the participants into low and middle liver fat groups using an additional cutoff at 2.5%.

In the Belly Fat study, liver fat content was assessed at baseline and after 12 weeks by ^{1}H-MRS using a similar method as in the NEO study described above. Details have been reported previously [27]. Participants were categorized into the same three liver fat groups as in the NEO study based on the preintervention data: low liver fat group ($n = 25$), middle liver fat group ($n = 24$) and high liver fat group ($n = 31$).

Metabolomics profiling for fasting, postprandial and postprandial response

Metabolite concentrations were quantified by the Nightingale high-throughput nuclear magnetic resonance (NMR) metabolomics platform (Nightingale Health Ltd., Helsinki, Finland) [28] in both the NEO study and the Belly Fat Study. This platform provides quantitative data on metabolites, including 14 lipoprotein subclasses, their lipid concentrations and composition, apolipoprotein A-I (ApoA) and B (ApoB), major FAs, BCAAs and AAAs, glycolysis-related measures, and ketone bodies. Additionally, it provides lipoprotein sizes (VLDL, LDL, and HDL diameter) and relative metabolite measures (i.e. percentages, ratios). The plasma samples are handled in 96-well plates, every plate containing 2 quality control samples, a plasma sample mimic and a mixture of 2 low-molecular–weight metabolites. The former is used to monitor the consistency of quantifications, whereas the latter is a technical reference to monitor the performance of the automated liquid handler and the spectrometer. Prior to data release, all metabolite measurements underwent standardized quality control procedures performed by Nightingale Health Ltd., more details have been reported previously [28].

In the NEO study, a total of 229 metabolite measures were available, including 148 absolute plasma metabolite concentrations and 81 ratios of metabolite measures. All metabolites were measured in fasting (T_0) and postprandial (T_{150}) plasma samples respectively. Postprandial metabolite responses Δ (T_{150}-T_0) were defined as postprandial minus corresponding fasting metabolite concentrations.

Similarly, in the Belly Fat study, 225 metabolite measures were quantified in plasma samples from T = 0, 120, 240 and 360 min after consumption of a high-fat, high-glucose liquid mixed meal, both before and after the interventions, by the Nightingale platform, of which 223 were the same measures as in the NEO study. Among

225 metabolite measures in the Belly Fat study, 146 are absolute plasma metabolite concentrations, and 79 are ratios of metabolite measures. The net incremental area under the curve (iAUC) was calculated using the trapezoid method [29] and used to define the postprandial response.

In both studies, metabolites with values that were not in the range of mean ± 5 * standard deviation (SD) were defined as outliers and were removed. Fasting and postprandial metabolite concentrations and postprandial metabolite responses (deltas and iAUCs) were log2-transformed to improve normality and reduce the influence of extreme values and Z-scored to allow for direct comparisons of effect sizes. A complete list of metabolites in both studies is shown in Supplemental Table 1.

Covariates information

In the NEO study, demographic characteristics information, such as age, sex, BMI, self-reported ethnicity (White/other), education (high/other), smoking status (ever/never), and menopausal status (postmenopausal yes/no) was collected using a questionnaire. Lipid-lowering medication (yes/no), glucose-lowering medication (yes/no), and anti-hypertensive medication (yes/no) were collected during the baseline visit. Total body fat (TBF, %) was estimated with the Tanita bioimpedance balance (TBF-310, Tanita International Division, United Kingdom). Habitual dietary intake of nutrients and alcohol intake (g/day) were estimated using a semi-quantitative 125-item food frequency questionnaire (FFQ) [30], and the Dutch Healthy Diet Index [31] (DHDI) was calculated as described previously [32]. Habitual physical activity during leisure time was assessed using the Short Questionnaire to Assess Health-enhancing physical activity(SQUASH [33]), which was expressed in hours per week of metabolic equivalents (MET h/week). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the formula: (fasting glucose * fasting insulin) / 22.5.

In the Belly Fat study, the information on age, sex, and BMI was collected during the visit both before and after the intervention.

Statistical analysis

In the NEO study, individuals with a BMI ≥ 27 kg/m^2 or higher were overrepresented [34]. To correct for this oversampling, all analyses were weighted towards the BMI distribution of participants from the Leiderdorp municipality, whose BMI distribution was similar to the BMI distribution of the general Dutch population [35, 36]. Therefore, the results are applicable to a general middle-aged Dutch population.

We first examined the associations between liver fat content and metabolite levels in fasting, postprandial states, and postprandial metabolite response in the NEO study. Baseline characteristics were expressed as proportions for categorical variables, and continuous variables following a normal distribution were expressed as mean and standard deviation (SD), while non-normally distributed variables were expressed as median and interquartile range (IQR), both for the total study population and three liver fat groups. We conducted linear regression analyses using liver fat content as both a categorical (high, middle, and low [reference]) and continuous variable for the exposure, adjusting for age, sex, BMI, TBF, alcohol intake, lipid-lowering medication, glucose-lowering medication, anti-hypertensive medication, self-reported ethnicity, education, smoking status, physical activity and DHDI. To account for multiple testing, p-values were adjusted using the Benjamini-Hochberg method to control the false discovery rate (FDR) at 0.05 across all metabolites jointly [37]. As a sensitivity analysis, we additionally adjusted for HOMA-IR to investigate whether the associations between liver fat groups and metabolite levels were independent of IR. The *svyglm* (survey generalized linear model) function of the *survey* R package was used to perform the analyses [38].

Baseline characteristics in the Belly Fat Study were compared between the high and low liver fat groups using an independent t-test for normally distributed numerical data and using Fisher's exact test for categorical data. First, in the Belly Fat Study before the intervention, we examined the associations between liver fat groups and metabolite levels of metabolites that were identified as significant in the NEO study using linear regression, with the low liver fat group as the reference group. The models were adjusted for age, sex, and BMI, with all intervention arms combined. Second, we examined whether intervention-induced Δliver fat was associated with changes in metabolites identified as significant in both studies. For this, we tested the associations between the Δliver fat (post-intervention - pre-intervention) and the Δfasting metabolites and also associations between the Δliver fat and the Δpostprandial response (post-intervention postprandial response [iAUCs] minus pre-intervention postprandial response [iAUCs]) using linear regression, adjusting for age, sex, ΔBMI and the intervention arm. Furthermore, we also tested the Pearson correlations between the Δliver fat and the Δmetabolites. The R function *lm* was used to perform the analyses [39].

To further explore whether sex modifies the associations between liver fat groups and plasma metabolite levels, we tested the interaction between sex and continuous liver fat on metabolite levels and subsequently performed sex stratified analysis in the NEO study using the *svyglm* function from the *survey* R package. More details are shown in the Supplementary Methods.

All analyses were performed in R version 4.4.1.

Results

Baseline characteristics

The baseline characteristics of the NEO study population, including BMI, fasting glucose, insulin, triglycerides, HOMA-IR, waist-to-hip ratio, and total body fat, are shown in Table 1. All participants ($n = 1986$) had a mean age of 56 years, and 53% were women. Among the 1,986 participants from the NEO study, 28.6% had low liver fat content (< 2.5%), 24.7% had middle liver fat content (2.5–5.5%), and 46.7% had high liver fat content (> 5.5%). The mean age of the participants was 56 years, and 53.0% were women. The baseline characteristics of participants from the Belly Fat Study are shown in Supplemental Table 2.

The associations between liver fat content and fasting as well as postprandial plasma metabolite concentrations in the NEO study

After multiple testing correction (FDR p-value < 0.05), compared to the low liver fat group, high liver fat group showed differences in 162 fasting metabolites, 173 postprandial metabolites and 152 postprandial metabolite

Table 1 Baseline characteristics of the participants from the NEO study with measurements of liver fat content and metabolomics ($n = 1986$)

	Total	Low liver fat (< 2.5%) (28.6%)	Middle liver fat (2.5%–5.5%) (24.7%)	High liver fat (> 5.5%) (46.7%)
Demographics				
Age (years)	56 ± 6	54 ± 6	56 ± 6	57 ± 6
Sex (women, (%))	948 (47.7)	369 (65.0)	233 (47.6)	346 (37.3)
Post-menopause, (%)	579 (61.1)	189 (51.2)	141 (60.5)	249 (80.0)
Education (high), (%)	746 (37.9)	219 (38.9)	186 (38.3)	341 (37.1)
Ethnicity (White), (%)	1907 (96.1)	541 (95.4)	471 (96.3)	895 (96.4)
Clinical / Metabolic characteristics				
BMI (kg/m^2)	25.9 ± 4.0	24.1 ± 3.1	26.5 ± 3.5	28.4 ± 4.1
WHR (%)				
Total	0.88 ± 0.08	0.8 ± 0.1	0.90 ± 0.1	0.9 ± 0.01
Men	0.94 ± 0.06	0.91 ± 0.05	0.94 ± 0.06	0.97 ± 0.06
Women	0.84 ± 0.07	0.81 ± 0.06	0.86 ± 0.07	0.89 ± 0.06
TBF (%)				
Total	30.8 ± 8.3	29.8 ± 7.8	30.5 ± 8.6	32.6 ± 8.6
Men	24.6 ± 5.5	21.5 ± 4.4	24.5 ± 4.4	27.4 ± 5.5
Women	36.2 ± 6.4	33.8 ± 5.6	38.6 ± 5.7	41.3 ± 5.3
Liver fat content (%)				
Total (mean ± SD) (median (IQR))	5.7 ± 7.8 2.6 (1.3, 6.2)	1.3 ± 0.5 1.3 (0.9, 1.8)	3.7 ± 0.9 3.5 (3.0, 4.4)	14.3 ± 10.0 10.9 (7.1, 17.7)
Men (mean ± SD) (median (IQR))	6.9 ± 8.0 3.7 (1.9, 8.3)	1.5 ± 0.5 1.6 (1, 1.9)	3.7 ± 0.9 3.5 (3, 4.2)	13.8 ± 9.1 10.9 (7.1, 17.1)
Women (mean ± SD) (median (IQR))	4.6 ± 7.5 1.8 (1.1, 4.5)	1.3 ± 0.5 1.2 (0.8, 1.6)	3.7 ± 0.9 3.6 (2.9, 4.5)	15.0 ± 11.4 11 (7.1, 19)
HOMA-IR	2.4 ± 2.5	1.7 ± 1.5	2.3 ± 1.5	3.7 ± 3.6
Plasma glucose (mmol/L)	5.4 ± 1.0	5.1 ± 0.6	5.5 ± 0.8	5.9 ± 1.4
Serum insulin (mU/L)	9.6 ± 7.5	7.2 ± 5.9	9.4 ± 5.4	13.6 ± 9.4
Serum TG (mmol/L)	1.3 ± 0.8	0.9 ± 0.5	1.3 ± 0.7	1.8 ± 1.1
Medication use				
Lipid-lowering medication (yes), (%)	274 (13.8)	46 (8.1)	62 (12.7)	166 (17.9)
Glucose-lowering medication(yes), (%)	84 (4.2)	6 (1.1)	15 (3.1)	63 (6.8)
Anti-hypertensive medication (yes), (%)	544 (27.4)	100 (17.6)	123 (25.1)	321 (34.6)
Lifestyle factors				
DHDI	71.3 ± 14.7	73.2 ± 15.0	72.0 ± 14.0	67.6 ± 14.1
Alcohol intake (g/day), median(IQR)	10.2 (2.6, 21.3)	8.0 (2.3, 16.9)	11.2 (2.2, 23.3)	13.6 (3.5, 28.7)
Smoking status (never), (%)	722 (36.4)	246 (43.3)	178 (36.3)	298 (32.1)
PA (MET h/week), median(IQR)	31.0 (16.0, 51.5)	32.8 (18.8, 53.5)	30.0 (16.5, 52.0)	26.8 (13.3, 46.0)

Results were based on analyses weighted toward the BMI distribution of the general population ($n = 1986$; 1038 men and 948 women). Numerical data are presented as mean (SD) if normally distributed and median (25th percentile, 75th percentile) if not. Liver fat content is presented as both mean (SD) and median (25th percentile, 75th percentile). Categorical data are presented as n (%). Abbreviations: BMI: Body Mass Index; WHR: waist to hip ratio; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; MET: metabolic equivalents; DHDI: Dutch Healthy Diet Index; TBF: total body fat.

responses (Fig. 2). Among them, 99 overlapped across all three states, while 146 were shared by fasting and postprandial states. Additionally, 49 were different between the high and low liver fat groups exclusively in the postprandial state and/or postprandial response (Fig. 3A).

Among the 162 metabolites that differed between the high and low liver fat groups at fasting state, the majority were lipoproteins. Compared to the low liver fat group, VLDL particles, VLDL-cholesterol, VLDL size, intermediate-density lipoprotein (IDL) particles, small LDL particles, and S-HDL-P, and ApoB were higher in the high liver fat group, while very-large and large HDL particles, HDL cholesterol and HDL size were lower in the high compared to the low liver fat group. In addition, the high liver fat group exhibited higher levels of total triglycerides, total FAs, SFAs, MUFAs, PUFAs, DHA, and omega-3 FAs compared with the low liver fat group, whereas the ratios of unsaturated FAs (omega-6%, PUFA% and LA%) were lower in the high compared to the low liver fat group. The amino acid alanine, the BCAAs (valine, leucine, isoleucine) and AAAs (phenylalanine and tyrosine) were also higher in the high compared to low liver fat group. GlycA was higher while acetate was lower in the high compared to the low liver fat group. Furthermore, most of the VLDL-related measures, including VLDL particle concentration, VLDL lipoproteins, VLDL cholesterol, and VLDL-triglycerides, were consistently higher in individuals with higher liver fat across fasting, postprandial levels, and postprandial responses (Fig. 2, Supplemental Table 3).

Among the 49 metabolites that differed between the high and low liver fat groups exclusively in the postprandial state and/or postprandial response, lipoprotein-related differences were primarily observed in LDL subfractions and cholesterol content of specific lipoprotein subclasses. For instance, total LDL-C decreased postprandially in the high liver fat group, while it increased in the low liver fat group (Supplemental Table 4). The FAs (linoleic acid and omega-6) increased postprandially and were higher in the high compared to low liver fat group at postprandial levels, while their postprandial responses were not different. Both the postprandial levels and postprandial responses of the amino acids (glutamine and histidine) were lower in the high compared to low liver fat group (Fig. 2, Supplemental Table 4). Furthermore, most of these differences across fasting, postprandial, and postprandial response states showed a dose-response pattern from low to middle to high liver fat group (Supplemental Table 3). For example, VLDL particles, VLDL-cholesterol, triglycerides, and most fatty acids (including SFA and LA) were higher in the high liver fat group than in the low live fat group. In contrast, measures related to

Fig. 2 Plasma metabolites in the high liver fat group compared to the low liver fat group. Results were based on analyses weighted toward the BMI distribution of the general middle-aged population ($n = 1986$; 1038 men and 948 women), and were derived from regression coefficients with 95% confidence intervals from linear regression analyses and expressed as standardized mean Differences in metabolites between the high and low liver fat groups, with the low liver fat group as the reference group, and adjusted for age, sex, BMI, TBF, alcohol intake, lipid-lowering medication, anti-diabetic medication, anti-hypertensive medication (yes/no), self-reported ethnicity, education (high/other), smoking status (ever/never), physical activity during leisure time and Dutch Healthy Diet index. *P*-values were adjusted for a false discovery rate (FDR) of 0.05 using the Benjamini-Hochberg method. The red dots indicate fasting metabolites (T_0), the green dots indicate postprandial metabolites (T_{150}), and the black dots indicate postprandial metabolite responses(T_{150}-T_0). The closed dots indicate FDR < 0.05, and the open dots indicate FDR ≥ 0.05

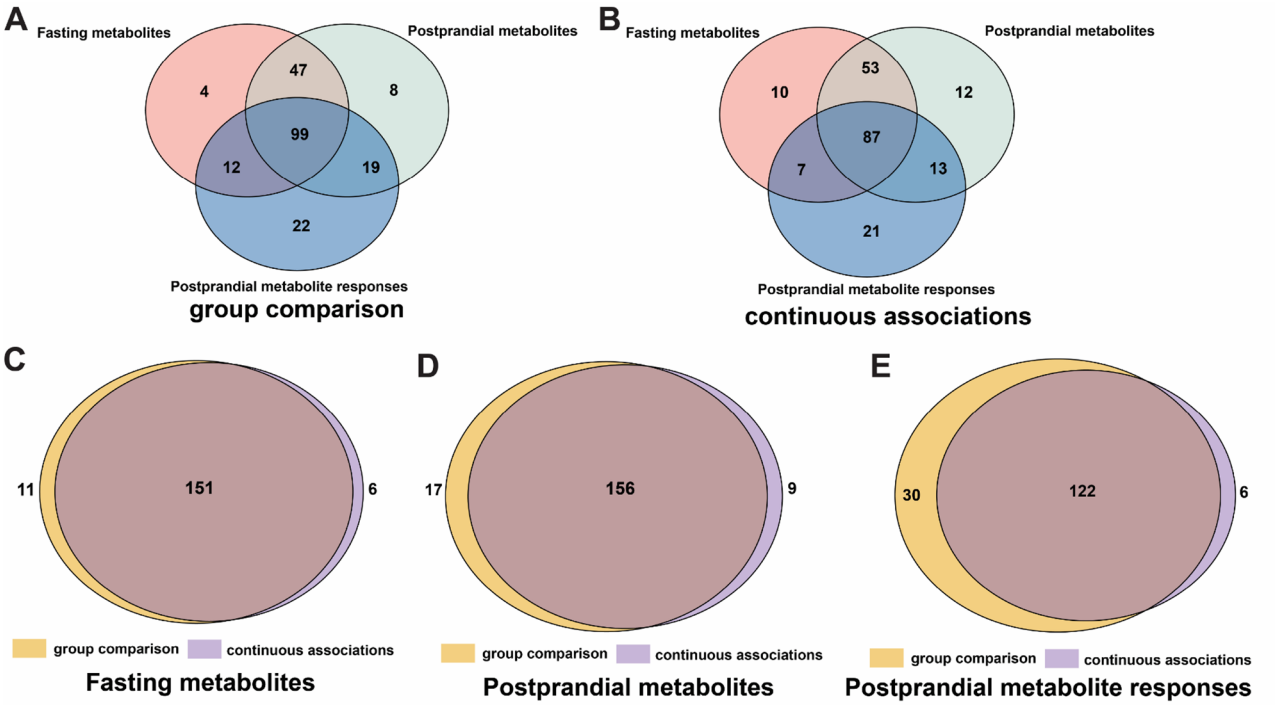


Fig. 3 Venn diagrams. **A** Venn diagram of the number of metabolites different between the high and low liver fat groups at fasting, postprandial and in postprandial responses. **B** Venn diagram of the number of metabolites associated with continuous liver fat content at fasting, postprandial and in postprandial responses. **C** Venn diagram of the number of metabolites different between the high and low liver fat groups and metabolites associated with continuous liver fat content at fasting. **D** Venn diagram of the number of metabolites different between the high and low liver groups and metabolites associated with continuous liver fat content at postprandial. **E** Venn diagram of the number of metabolites different between the high and low liver fat groups and metabolites associated with continuous liver fat content in postprandial responses

larger HDL particles, such as very large and large HDL, demonstrated the opposite pattern, with levels progressively decreasing across increasing liver fat categories.

We then additionally examined the associations between continuous liver fat content and plasma metabolite levels. The findings were consistent with the results of the group comparisons at fasting, postprandial and in postprandial responses (Fig. 3C-E). Details of the findings are shown in Supplemental results and Supplemental Table 3.

Sensitivity analyses were performed by further adjusting for HOMA-IR, using liver fat content as categorical data. Results showed that 155 fasting metabolites, 167 postprandial metabolites, and 143 postprandial metabolite responses differed between the high and low liver fat groups, which largely overlapped with the findings in the main model (Supplemental materials, Supplemental Table 3). The absolute values of metabolic measurements across fasting, postprandial and postprandial response states in the NEO study are shown in Supplemental Table 5.

Fasting plasma metabolites and postprandial metabolite responses (iAUC) in the high versus low liver fat group in the Belly Fat Study

To validate our findings in an independent population, we compared pre-intervention fasting plasma metabolite levels between individuals with high and low liver fat in the Belly Fat Study, focusing on the 162 fasting metabolites identified in the NEO study, of which 157 were replicable in the Belly Fat Study. Among these 157 fasting metabolites, 71 also differed significantly between the high and low liver fat groups. Almost all showed differences in the same direction across studies, except for L-VLDL-CE%, which was higher in the high versus low liver fat group in the NEO study but showed the opposite pattern in the Belly Fat Study (FDR p-value < 0.05; Fig. 4, Supplemental Table 6).

We additionally compared pre-intervention postprandial metabolite responses (iAUC) for the 152 metabolites identified in the NEO study, of which 151 were measured in the Belly Fat Study. No significant differences in these postprandial metabolite responses were observed between the high and low liver fat groups after FDR correction (Supplemental Table 7).

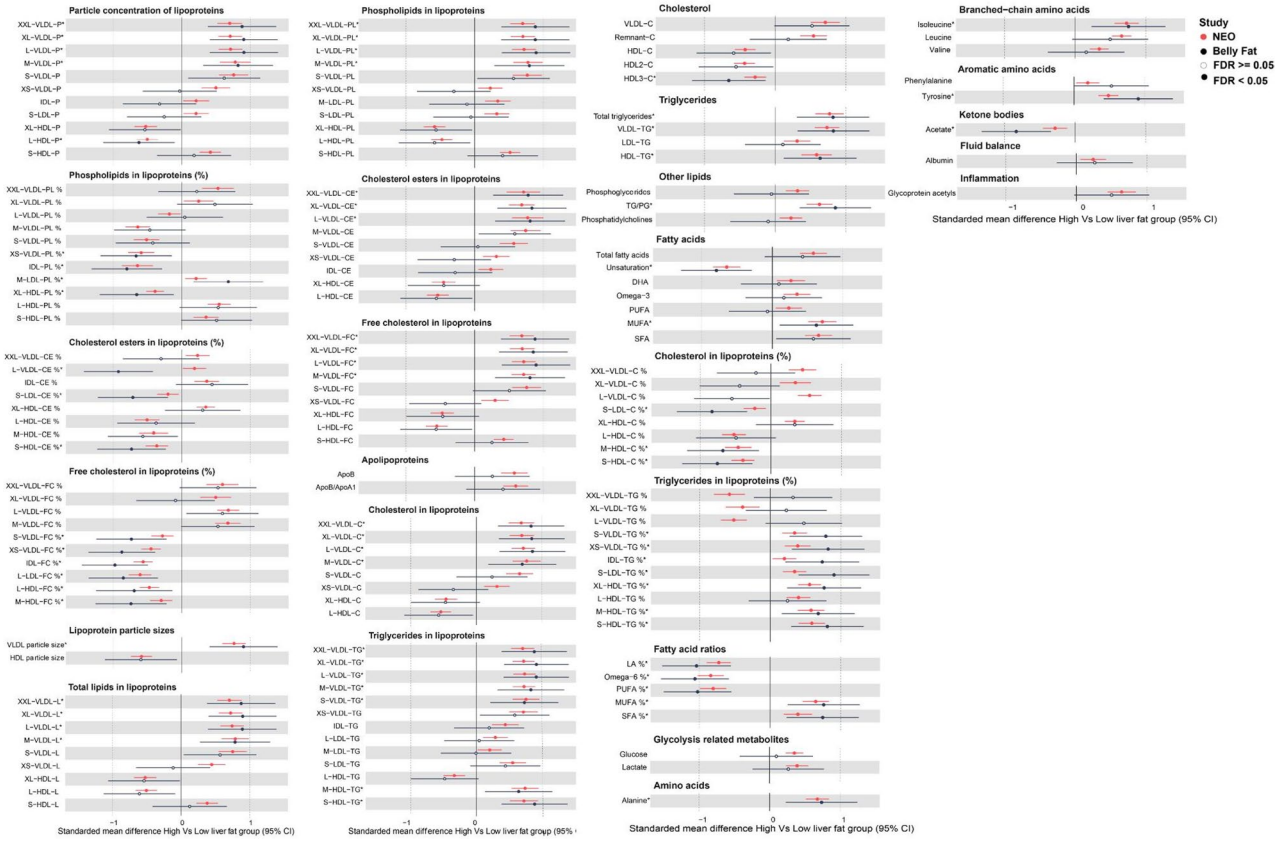


Fig. 4 Fasting plasma metabolites in the high compared to the low liver fat group in the Belly Fat Study and the NEO study, restricted to metabolites identified in the NEO study ($n = 1986$) and measured in the Belly Fat Study ($n = 80$). Differences between the high and low liver fat groups were tested using linear regression with adjustment for age, sex, BMI, TBF, alcohol intake, lipid medicine, glucose medicine, hypertensive medicine, ethnicity, education, smoking status, physical activity and DHDI in the NEO study, and age, gender, BMI were adjusted in the analyses in the Belly Fat Study. *P*-values were adjusted for a false discovery rate (FDR) of 0.05 using the Benjamini-Hochberg method. The red dots represent the comparison results for fasting metabolites in the NEO study, and the black dots represent the comparison results for fasting metabolites in the Belly Fat Study. The closed dots indicate FDR < 0.05, and the open dots indicate FDR ≥ 0.05. The 71 metabolites with asterisks indicate that they are different between the high and low liver fat groups in both the Belly Fat Study and the NEO study at the significant level of FDR < 0.05

Associations between diet-induced changes in liver fat content and changes in metabolite levels in the Belly Fat study

For the 71 fasting metabolites that were cross-sectionally associated with liver fat content in the Belly Fat and the NEO study, we further investigated the associations between continuous Δliver fat content and Δfasting metabolite levels after the 12-week intervention, with adjustment for age, sex and ΔBMI and intervention arms. The details of baseline and changes in liver fat content, body weight and BMI upon intervention in the Belly Fat Study are shown in Supplemental Table 8.

Continuous Δliver fat was associated with 40 out of the 71 Δfasting metabolites, with consistent directions as the cross-sectional associations between the liver fat content and these fasting metabolites identified in the NEO study (Fig. 5A, Supplemental Table 9). Out of the 40 metabolites, 24 of them were particle concentrations of XXL-, XL-, L-, and M-VLDL subclasses, along with their lipid content, and one was S-HDL-TG, all of which showed a positive association with Δliver fat. Additionally, six of them were lipoprotein fractions, relative lipid fraction (%) of XS-VLDL, L-LDL and IDL subclasses showed negative associations with Δliver fat, and relative triglycerides fraction of S-HDL and S-LDL showed positive associations with Δliver fat. Changes in the tyrosine and isoleucine were also positively associated with Δliver fat. Four metabolites were FAs or FAs ratios: SFA% and MUFA were positively associated with Δliver fat, while omega-6% and the degree of unsaturation in FAs were negatively associated with Δliver fat. The remaining three metabolite measures were total triglycerides, VLDL triglycerides and TG/PG, all of which were positively associated with Δliver fat (Fig. 5A-B). The Pearson correlations between Δliver fat and Δfasting plasma metabolites were also examined. These correlations aligned with the associations observed in the linear regression analysis. Metabolites positively associated with Δliver fat clustered

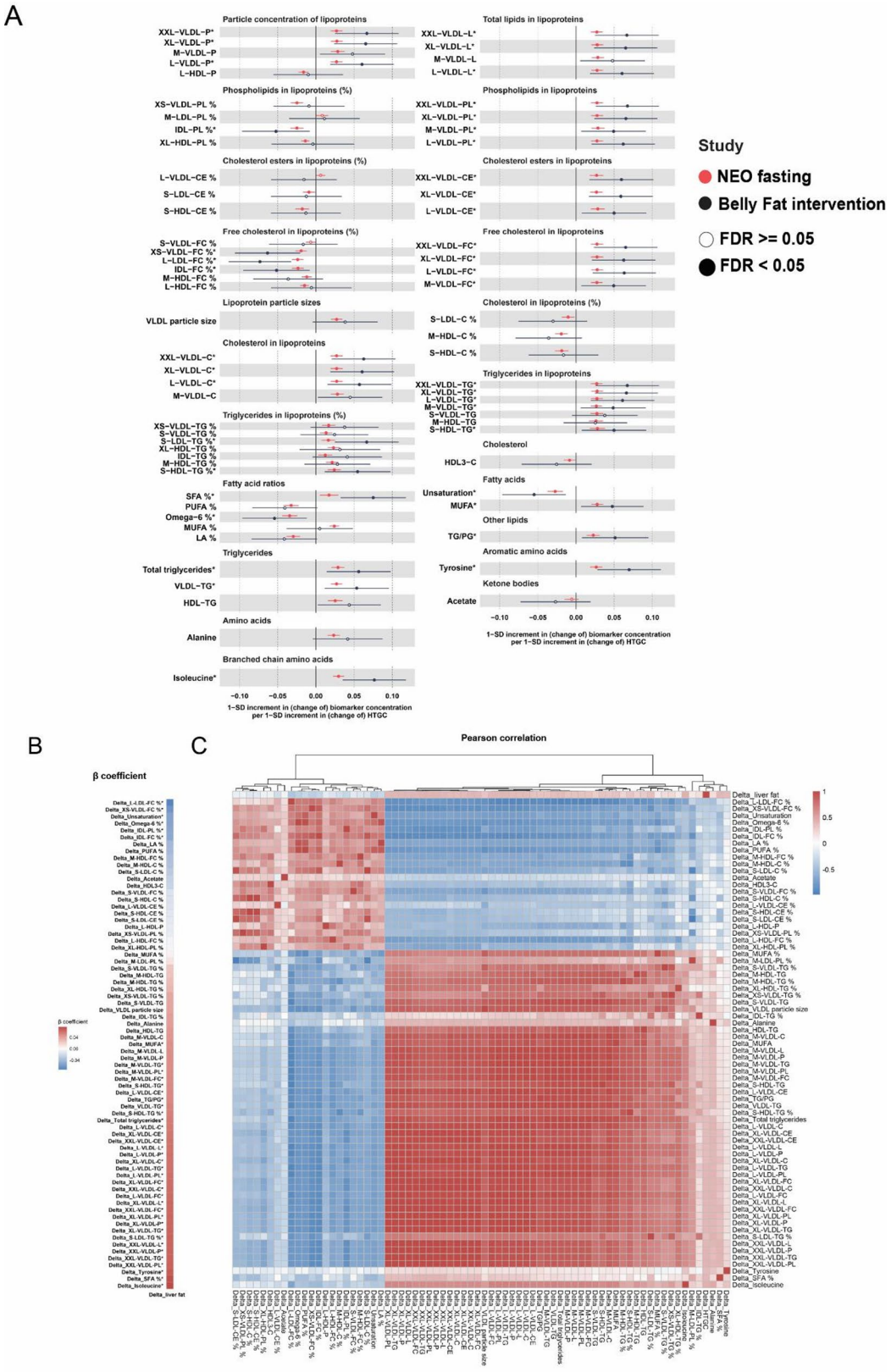


Fig. 5 (See legend on next page.)

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Fig. 5 Associations and correlations between Δliver fat and after intervention in the Belly Fat study, restricted to metabolites identified cross-sectionally in the NEO study ($n = 1986$). **A** Associations of the liver fat content and the fasting metabolites in the NEO study (cross-sectional) and the associations of the change of liver fat content and the change of fasting metabolites after 12 weeks in the Belly Fat Study. Associations between the change in liver fat and the change in fasting plasma metabolites after interventions in the Belly Fat Study (black dots) were tested using linear regression with adjustment for age, gender, change of BMI and diet. Cross-sectional associations between liver fat and fasting plasma metabolites in the NEO study (red dots) were tested using linear regression with adjustment for age, gender, BMI, TBF, alcohol intake, lipid medicine, glucose medicine, hypertensive medicine, ethnicity, education, smoking status, physical activity and DHDI. *P*-values were adjusted for a false discovery rate (FDR) of 0.05 using the Benjamini-Hochberg method. The closed dots indicate FDR < 0.05, and the open dots indicate FDR ≥ 0.05. The 40 metabolites with asterisks indicate that they significantly differ between the high and the low liver fat groups, both in changes after intervention in the Belly Fat Study and fasting levels in the NEO study. **B** Heatmap of associations between the change in liver fat content and the change in fasting plasma metabolites after interventions in the Belly Fat Study, the 40 metabolites with asterisks indicate that they significantly differ between the high and the low liver fat groups both in changes after intervention in the Belly Fat Study and fasting levels in the NEO study. **C** Heatmap of the correlations between the Δmetabolites and Δliver fat after interventions in the Belly Fat Study, the correlations were tested using the Pearson method

together, while those negatively associated with Δliver fat formed a separate cluster (Fig. 5C). However, no associations were found between the diet-induced Δliver fat and the Δpostprandial responses (iAUCs) after the FDR correction (Supplemental Table 10).

Sex-specific associations between liver fat content and metabolite levels in the NEO study

We assessed potential effect modification by sex in the associations between liver fat content and fasting, postprandial metabolites, as well as metabolite postprandial responses, by testing interactions between continuous liver fat content and sex using linear regression in the NEO study. The associations of liver fat content and 56 fasting plasma metabolites were modified by sex after FDR correction, the majority of which were S-VLDL, XS-VLDL, all LDL subclasses, XL and L-HDL subclasses. Additionally, the associations of liver fat content and HDL particle size, LDL cholesterol and ApoB/ApoA ratio were also modified by sex (Fig. 6A). Only seven postprandial metabolites were differently associated with liver fat between men and women (Fig. 6B). Except for 3-hydroxybutyrate and L-VLDL-C%, the other five were also found at fasting (Supplemental Table 3). No postprandial metabolite responses were found to be modified by sex. We further investigated the differences in these metabolites between the high and low liver fat groups. Most of the differences in these metabolites between the high and low liver fat groups were more pronounced in women than in men (Fig. 6). Sex-specific descriptive characteristics for age, BMI, HOMA-IR, plasma glucose, serum insulin, and serum triglycerides in the whole population and each liver fat group are presented in Supplemental Table 11.

Discussion

In this study, we integrate data from an observational cohort and a dietary intervention study to examine the associations of liver fat content and changes in liver fat with fasting and postprandial plasma metabolite profiles. We uncovered 49 associations exclusively between liver fat content and postprandial metabolites/response. We also identified metabolites that changed in tandem with diet-induced alterations in liver fat after adjusting for BMI change. Cross-sectional associations from the cohort study (i.e., the NEO study) aligned with associations between diet-induced (changes in) liver fat and fasting metabolites in the intervention study (i.e., the Belly Fat Study), but not with changes in postprandial responses.

Previous research in the UKB has examined the association between liver fat content and plasma metabolite levels [11]. Although UKB participants were in random non-fasting states, our findings based on fasting metabolites are largely consistent with the UKB results. We observed that elevated liver fat content was associated with higher fasting levels of larger VLDL subclasses, triglycerides, ApoB/ApoA, various FAs (including SFA, MUFA, PUFA, DHA, and omega-3), BCAAs and AAAs, GlycA, larger VLDL particle size, and smaller HDL size, compared to individuals with low liver fat. Furthermore, in individuals with elevated liver fat, MUFA and SFA% were higher, while PUFA% was lower, compared to individuals with low liver fat. These results align with reported associations between liver fat and plasma metabolites in the UK Biobank [11]. Furthermore, most of the differences in VLDL subclasses and VLDL size were consistent across fasting, postprandial levels, and postprandial responses. The extent of liver steatosis has been positively linked to both the size and number of VLDL particles produced [40]. VLDLs are synthesized and released by the liver into the bloodstream, playing a key role in delivering triglycerides and cholesterol to peripheral tissues [41, 42]. In addition, HDL particle size has been suggested as an indicator of the efficiency of reverse cholesterol transport. Smaller HDL particles are thought to infiltrate the interstitial space more freely, where the majority of peripheral cell cholesterol removal occurs [43, 44]. The higher levels of VLDL subclasses, larger VLDL size and smaller HDL particle size found in individuals with elevated liver fat across fasting, postprandial levels, and postprandial responses in this study indicate increased hepatic VLDL production and less capacity for cholesterol removal associated with

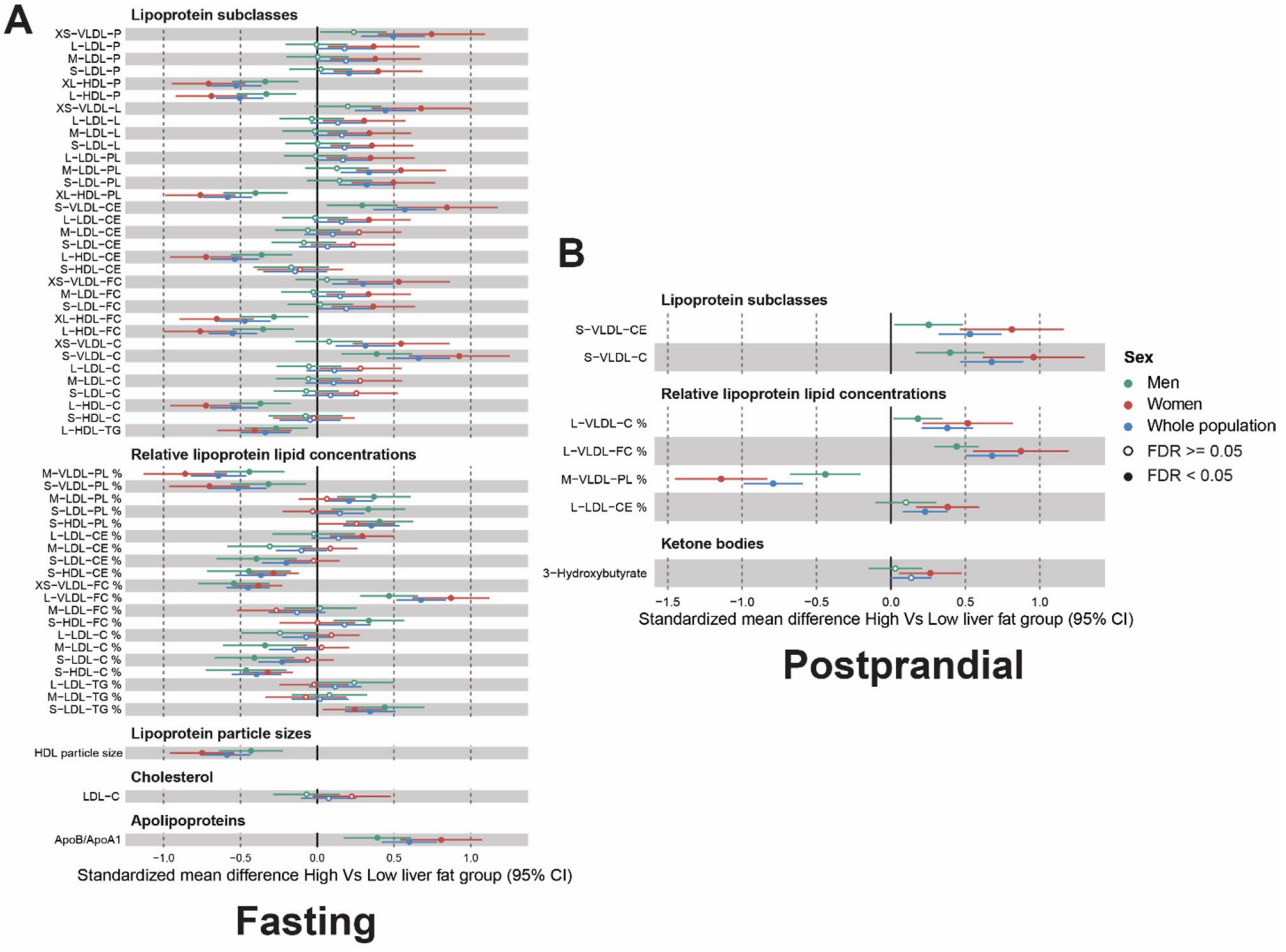

Fig. 6 Sex-specific plasma metabolites in the high liver fat group compared to the low liver fat group in the NEO study. **A** Differences in fasting plasma metabolites between the high and low liver fat groups in men, women and the whole population. **B** Differences in postprandial plasma metabolites between the high and low liver fat groups in men, women and the whole population. The differences were tested using linear regression with adjustment for age, sex, BMI, TBF, alcohol intake, lipid medicine, glucose medicine, hypertensive medicine, ethnicity, education, smoking status, physical activity, DHDI and menopausal status. *P*-values were adjusted for a false discovery rate (FDR) of 0.05 using the Benjamini-Hochberg method. The green dots represent the comparison results in men, the red dots represent the comparison results in women, and the blue dots represent the comparison results in the complete population. The closed dots indicate FDR < 0.05, and the open dots indicate FDR ≥ 0.05

elevated liver fat, which is already evident in the fasting state and persists postprandially. The higher postprandial responses further indicate that, although these differences were already present in the fasting state, they became more pronounced following meal ingestion, suggesting potential impaired lipoprotein handling in individuals with elevated liver fat. This interpretation can be supported by the higher ApoB/ApoA ratio observed across fasting, postprandial levels, and postprandial responses in the current study, which has been strongly associated with increased risk of myocardial infarction, stroke, and other cardiovascular manifestations, as ApoB reflects the number of potentially atherogenic lipoprotein particles whereas ApoA represents anti-atherogenic HDL particles [45–47].

A potential mechanism for the elevated proportion of SFA might be explained by increased hepatic de novo lipogenesis in individuals with higher liver fat. The resulting rise in SFA availability may subsequently promote desaturation into MUFA, consistent with the higher MUFA proportions observed in our study [48, 49]. The observation of higher absolute PUFA alongside lower PUFA% in individuals with high compared to low liver fat likely reflects a compositional effect, indicating that with increasing liver fat, the overall circulating fatty acids pool expands, leading to higher absolute concentrations of multiple fatty acids, including PUFA. While other fatty acids, such as SFA and MUFA, increase proportionally more strongly than PUFA, this reduces the relative contribution of PUFA and may explain the negative

association of PUFA% despite the positive association of absolute PUFA concentrations with liver fat.

Many of the metabolite associations observed in relation to higher liver fat, such as higher levels of VLDL particles, triglycerides, BCAAs, and GlycA, are also reported to be associated with higher insulin resistance [50, 51]. Importantly, in our data, most associations remained significant after adjustment for HOMA-IR, suggesting that liver fat contributes to these metabolic associations beyond insulin resistance alone. Nevertheless, residual confounding by insulin resistance can never be entirely excluded [52].

Postprandial dysmetabolism has emerged as a cardiovascular risk factor independent of fasting metabolism [53]. In terms of metabolites that differed exclusively in the postprandial state and/or postprandial responses, the high liver fat group showed lower responses of medium and large LDL particles and their lipid fractions, as well as the cholesterol content of small LDL, IDL, and small HDL subclasses, and the relative cholesterol content (%) of XS-MVLDL. In the fasting state, increased liver fat is known to stimulate hepatic VLDL overproduction, which likely explains why the strongest associations were observed at the level of VLDL particles [41]. Postprandial triglyceride-rich lipoproteins metabolism is closely coupled with LDL and HDL metabolism [54]. Exchange of core lipids between postprandial LDL/HDL is increased during prolonged lipemia via CETP-mediated mechanisms, resulting in small, dense LDL particles and reduced HDL cholesterol levels [54]. Thus, while fasting findings primarily reflect hepatic lipid overproduction, postprandial alterations may reflect impaired TRL clearance and lipoprotein remodeling. Consistent with reports that LDL-C decreased postprandially compared with fasting in T2D patients [55, 56], we observed decreases in LDL particles, LDL-cholesterol, and small HDL subclasses in individuals with high liver fat, whereas these measures increased in those with low liver fat. These indicate different metabolic flexibility in response to meal ingestion in individuals with elevated liver fat, suggesting that the postprandial lipoprotein profile in the high liver fat group resembles the dyslipidaemic response reported in T2D, providing insight into metabolic alterations associated with increasing liver fat and potential CVD risk.

Postprandial levels of glutamine, histidine, omega-3%, DHA%, ApoA1, and sphingomyelins were lower in the high liver fat group compared with the low liver fat group, whereas omega-6% and LA% were higher. These differences were not observed at fasting state, indicating that individuals with differing liver fat levels respond differently to meal ingestion with respect to these metabolites. Interestingly, the previous findings from a subset of the NEO study showed lower levels of postprandial histidine and glutamine in individuals with T2D compared to healthy individuals [57]. Our findings indicate that the dysregulation of postprandial metabolism of these amino acids may have already started at the stage of increasing liver fat prior to the onset of metabolic disease. However, the postprandial state is a dynamic, nonsteady-state condition in which humans spend the majority of time [58]. As our postprandial measurement was obtained at a single time point (2.5 h), this limits our ability to capture the temporal dynamics of postprandial lipid metabolism fully. Nevertheless, our findings provide information on liver fat–related postprandial lipoprotein metabolism beyond the fasting state. Future large studies with multiple time points are therefore warranted to better delineate these dynamic responses and their implications for health and disease.

The lipoprotein profile we observed in the high versus low liver fat group is commonly reported in obesity and T2D, and reflects a highly atherogenic pattern associated with increased CVD disease risk [59, 60]. Our findings show that this lipid profile is specifically related to higher liver fat; therefore, individuals with higher liver fat may be at increased risk of developing cardiometabolic disease, compared to individuals with low liver fat.

To confirm potential causal links between liver fat content and metabolites, we additionally explored the associations between Δliver fat and Δfasting metabolites upon a 12-week dietary intervention in this independent study. Interestingly, reductions in liver fat were accompanied by decreases in most of the VLDL lipoproteins, triglycerides, and BCAAs at fasting state. The directions of these associations were consistent with the associations found in the cross-sessional analyses. The fasting XXL-, XL-, L-, and M-VLDL subclasses related metabolites, as well as tyrosine and isoleucine, were reported to be associated with liver fat [41, 61]. The concordance of these associations at both the cross-sectional and Δ (change) levels provides strong evidence that these metabolites are robustly related to liver fat. In the disease-wide association studies in UKB, these metabolites were also strongly associated with type 2 diabetes [62], suggesting that reducing liver fat may lower these metabolite levels, thereby potentially decreasing the risk of chronic conditions such as type 2 diabetes. However, we could not find any associations between Δliver fat and Δpostprandial metabolite responses. The limited sample size can be a potential reason limiting the ability to detect such associations, particularly given the high inter-individual variability in postprandial metabolic responses. In addition, differences in the assessment of postprandial responses due to the distinct postprandial sampling schemes used in the two studies may have further limited the comparability of postprandial findings between studies.

Sex differences in lipid metabolism are widely recognized [63, 64]. We observed a greater number of fasting

liver fat-metabolite associations, as well as larger effect estimates, in women than in men previously [1]. In the current study, 56 fasting plasma metabolites showed sex-specific associations with liver fat, the majority of which were lipoproteins and their relative proportions in the cohort study. Interestingly, these associations were more pronounced in women compared to men after adjustment for menopausal states. Although women generally display a more favourable plasma lipid profile than men, several studies have shown that under conditions of impaired metabolic health, such as obesity or type 2 diabetes, women may exhibit greater disturbances in lipid and lipoprotein metabolism than men [65, 66]. Other factors, i.e. total body fat, visceral fat accumulation, and insulin-sensitivity, also play a role in the sexual dimorphism of lipid metabolism [67]. Lipid metabolism in women is influenced by sex hormones and menopausal status, which may contribute to sex differences in metabolic profiles associated with liver fat. For example, LDL-C levels are known to increase during the menopausal transition [68]. In the current study, menopausal status and total body fat were both included as covariates in the analyses, suggesting that the observed sex-specific associations are unlikely to be explained solely by differences in menopausal status or body fat. Nevertheless, residual biological differences between men and women in these factors cannot be fully excluded, warranting further investigation. Interestingly, in the postprandial state, liver fat showed much less sex-specific associations. The disappearance of sex-specific associations postprandially suggests that meal ingestion may override sex effects on liver fat-metabolite associations. Consistent with this, a more pronounced link between liver IR and postprandial lipid profile in women compared to men was also observed previously, although the underlying mechanisms remain unclear [69]. These observed sex differences in the relationship between various liver fat and metabolic profiles underscore the importance of accounting for sexual dimorphism, and further mechanistic studies are needed to elucidate how sex-related hormonal shifts influence metabolite profiles and hepatic lipid metabolism at both fasting and postprandial states.

Strengths and limitations

The major strength of this study is the extensive metabolite profiling in both fasting and postprandial states and the HTCG measurements by ^{1}H-MRS. Furthermore, the large sample size in the cohort increased the statistical power of the findings, and validation through an intervention study further strengthens the robustness of the results and identifies novel information on metabolites that change with alterations in liver fat content. However, several limitations should also be acknowledged. First, postprandial metabolite measurements in the NEO study were limited to 2.5 h after the liquid mixed meal. Moreover, likely due to the limited sample size of the intervention study and differences in postprandial sampling time points between the observational and intervention studies, postprandial findings could not be replicated across the two studies. Second, the range of metabolites measured by the Nightingale platform is limited compared with untargeted metabolomic approaches. Therefore, further postprandial studies with comparable mixed-meal compositions, consistent sampling time points, and broader metabolite coverage using other platforms in diverse populations are needed.

Conclusions

In conclusion, varying levels of liver fat content are associated with distinct metabolic profiles across both fasting and postprandial states. Postprandial metabolomics provides complementary insight into lipid and fatty acid metabolism in relation to liver fat beyond fasting measures. Diet-induced reduction in liver fat is accompanied by favorable metabolic changes at fasting. In addition, the observed sex-specific patterns further underscore the importance of considering sexual dimorphism when investigating liver fat–related metabolic profiles. Future studies with harmonized postprandial assessment are needed to further elucidate the postprandial observations and the underlying mechanisms.

Abbreviations

AAA	Aromatic amino acids
ApoA	Apolipoprotein A-I
ApoB	Apolipoprotein B
BCAA	Branched-chain amino acids
BMI	Body mass index
CVD	Cardiovascular diseases
DHA	Docosahexaenoic acid
DHDI	Dutch healthy diet index
ER	Energy restricted
FAs	Fatty acids
FFQ	Food frequency questionnaire
^{1}H-MRS	Proton magnetic resonance spectroscopy
HDL	High-density lipoprotein
HOMA-IR	Homeostatic model assessment for insulin resistance
iAUC	Incremental area under the curve
LDL	Low-density lipoprotein
LUMC	Leiden University Medical Center
MASLD	Metabolic dysfunction-associated steatotic liver disease
MET	Metabolic equivalent
MUFA	Monounsaturated fatty acids
NEO	The Netherlands epidemiology of obesity study
NMR	Nuclear magnetic resonance
PUFA	Polyunsaturated fatty acids
SD	Standard deviation
S-HDL-P	Small high-density lipoprotein particles
SQUASH	Short questionnaire to assess health-enhancing physical activity
TBF	Total body fat
T2D	Type2 diabetes
TG	Triglycerides
VLDL	Very-low-density lipoprotein
WHR	Waist-to-hip ratio

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12933-026-03158-4.

Supplementary Material 1

Supplementary Material 2

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Author contributions
YF, LAA and RL-G designed this study. YF, KD, and RL-G performed statistical analyses. YF drafted the manuscript. AG, RL-G, LAA and RdM participated in data interpretation and critically reviewed and revised the manuscript. HL, DOMK, and KWD were involved in the data collection. LAA, RL-G, RdM and AG supervised the research. LAA conducted the study design and data collection of the Belly Fat study. RdM and FRR conducted the study design and data collection of the NEO study. All authors revised the manuscript and approved the final version for publication.

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Data availability
The datasets used during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The main study protocol was approved by the medical ethics committee of Leiden University Medical Center (NL21981.058.08/P08.109) and the ethics committee of Wageningen University and Research (NCT02194504). All participants gave written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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